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THE RELATIONSHIP OF SERUM PHOSPHATASES TO SEX STEROIDS

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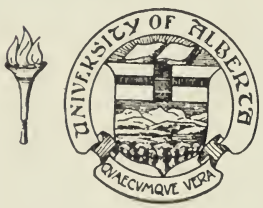

University of Alberta

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This is to certify that the undersigned have read and recommend to the Committee on Graduate Studies for acceptance, a thesis submitted by Donald B. Baker, B.Sc. entitled:

The Relationship of Serum Phosphatases To Sex Steroids

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THE RELATIONSHIP OF SERUM PHOSPHATASES TO SEX STEROIDS

by

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Research Fellow College of Physician's and Surgeons of Alberta

A thesis submitted in conformity with the requirements for the degree of Master of Science by the University of Alberta.

Department of Biochemistry
University of Alberta
1948

1948
#1

A C K N O W L E D G M E N T S

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Studies on the Hemoglobin, Hematocrit, Serum Protein, Serum Inorganic, Phosphorus and Serum Phosphatase Levels in Rats in Relation to Deficiencies of Members of the Vitamin B. Complex.

I. INTRODUCTION

In 1912 an enzyme was described by Grosser and Husler (17) in the intestinal mucosa and kidney which was capable of hydrolyzing phosphoric esters. Robison (37) found that a similar enzyme was particularly high in activity in growing bone and cartilage and its activity was greatest at pH 9 to 9.5. This alkaline enzyme was found by Kay (27) to increase in the serum in certain bone diseases including neoplastic metastases.

Davies (10) discovered a phosphatase with pH optimum of 4.8 in the spleen and kidney in addition to the alkaline enzyme. Kutscher and Wolbergs (34) found the acid enzyme to be highly active in the prostate and it was later shown by Gomori (15) that in the Rhesus monkey the enzyme occured in and was formed by the acinar epithelium of the prostate gland.

In 1938 the Gutmans (19) introduced the subject of sex hormones in relation to phosphatases. These authors investigated the alkaline and acid phosphatase of the prostate of rats after the injection of male and female hormones and showed no effect to occur on the acid enzyme and very little on the alkaline enzyme. Extending their work to include the Rhesus monkey the same authors (21) later showed that testosterone propionate caused a several hundred fold increase

in the acid enzyme of the prepubertal prostate to adult levels. They inferred in the same phenomenon occurred in man. This species difference between rat and monkey which they had shown to occur in relation to the injection of testosterone, could be accounted for by the fact, as they had previously shown (19), that the rat has a low concentration of acid phosphatase in the prostate. However the rat has a high source of acid phosphatase in the closely related preputial glands (19) and it would have been of interest to have seen whether any alteration occurred here on the injection of testosterone.

In carcinoma of the prostate with metatases to bone, both acid and alkaline enzyme activity are high at all foci of growth and in the serum (12)(18)(20). With this in view Huggins and Hodges (25) decided to test the effect of hormones on the serum enzymes under these conditions. They showed that castration and the administration of estrogens effect a rapid reduction in serum acid phosphatase. The alkaline enzyme showed an initial marked rise followed by a gradual decline. Androgens caused the opposite effect, producing a sharp rise after injection. This work was confirmed and extended by Sullivan (40)(41), Herger (23), Watkinson (43) and others. These observations led to the application of estrogen therapy and castration as an aid in relieving the pain and discomfort of carcinoma of the prostate and reducing

the rate of metastatic growth and spread (24).

Huggin's observations indicate that there is a rough correlation between the levels of serum acid and alkaline phosphatase and the level of male sex hormone. This conclusion is corroborated by Engberg (11) who found a correlation to exist between the level of androgen excretion in the urine and semen acid phosphatase in cryptorchid patients.

Other clinical observations are pertinent in this regard. Chronic cystic mastitis (hyperplasia and mastodynia) and gynecomastia are completely relieved by the administration of androgens and aggravated by estrogens. Recently Adair (1) among others presented evidence that the spread of breast cancer can be inhibited and in some instances regression in the malignant process produced by massive androgen therapy. The relationship of these changes to phosphatases has not yet been studied but it seems significant that in both cancer of the prostate and breast a prominent characteristic is spread of the metastases to bone.

Under experimental conditions only scanty work has been done on the relationship between the serum phosphatases and the various estrogens and androgens separately or in combination with castration. Huggins (25) using dogs found no alteration in the enzymes following castration. Buchwald (5)(6) found an increase in the acid phosphatase activity

on castration of both male and female rats. He extended his observations further by injection testosterone propionate into normal and castrated females and diethylstilboestrol into normal and castrated males. An increase in serum alkaline enzyme with no alteration in the acid enzyme was noted in the normal female. In the normal male he found no alteration in the alkaline enzyme but a decrease occurred in the acid enzyme. In both instances castration neither decreased nor magnified the results of the injections.

Since control therapy of carcinoma of the prostate has for its basis the maintenance of a low androgen and acid phosphatase level (22) it seemed important to have confirmation of Buchwald's work and to extend it to include more of the sex hormones in a greater number of combinations.

Rats were chosen as a suitable experimental animal with which to conduct this investigation since it had been previously shown that their serum alkaline phosphatase level was about 25 times the human level (9).

II. METHODS

1. The Experimental Animal

Adult male and female albino rats (Wistar strain) housed in all metal cages were used throughout the experiment. At 5 P.M. daily the animals were fed a mixture of Purina and Victor fox chows in excess of their daily requirements except when special feeding experiments were done. Tap water was supplied ad lib. The daily amount of food consumed was determined by feeding to a group of animals a weighed amount of checkers in excess of the normal daily requirement and weighing the uneaten food on the following 24 hour period. The resultant weight of food divided by the number of rats gave the average weight of checkers eaten per rat per day.

2. The Oestrous Cycle

Vaginal smears were made by inserting a rubber teated fine pipette containing a few drops of distilled water into the vagina, washing the vagina a number of times with the water and then smearing the vaginal secretion and water on a glass slide. The material on the slide was stained by the differential staining method of Shorr (39). This consists of:

(1) fixing the smear for 1 to 2 minutes while not quite dry in a solution of equal parts of ether and 95%

alcohol.

(2) staining 1 minute with the Shorr stain*

(3) blotting dry and carrying through 70% EtOH and 95% EtOH dipping 10 times in each solution and blotting dry.

(4) clearing the slide well in xylol.

Stained in this manner the cornified cells appear brilliant orange-red and the non-cornified cells blue-green. The determination of the stage of oestrous was then done by examining the stained smears and classifying them in accordance with a modification (26) of the method of Long and Evans (35) which is as follows:

Stage I. Epithelial cells only in large numbers, of characteristic appearance with the stained part drawn in leaving a white ring around the cell or part of it.

Stage II. Preponderance of cornified cells with a few leucocytes and epithelial cells.

Stage III. Cornified cells only.

Stage IV. Preponderance of leucocytes associated with a few epithelial and cornified cells.

Stage V. Preponderance of epithelial cells with a few cornified cells and leucocytes.

Heat occurs in the latter part of Stage II and the early part of Stage III.

* Fast green FCF, Biebrich scarlet, Orange G, Aniline blue, Phosphotungstic acid and Phosphomolybdic acid dissolved in ethyl alcohol to which glacial acetic acid has been added.

3. Castration

(a) Orchidectomy

A single midline incision was made in the scrotum just long enough to permit the extrusion of one of the testicles at a time. The internal spermatic vessels, the ductus deferens and its vessels were clamped to ensure hemostasis and the testis together with the epididymis was excised. The incision was closed by the use of nickel silver wound clips.

(b) Oophorectomy

A single longitudinal skin incision was made in the dorsal midline at the level of the lower poles of the kidneys. The skin was retracted laterally toward either side and the ovary was exposed by cutting through the thin muscle wall ventral to the dorsal muscle mass. The incision was long enough to allow extrusion of the ovary and part of the uterus. Hemostasis was insured by clamping the upper horn of the uterus and the ovary together with its surrounding fat, the oviduct and a small portion of the uterus were excised. The outer skin incision was closed with wound clips.

Male castrate rats were given at least 12 weeks for recovery before being used for sex steroid studies. Female castrates were given at least 8 weeks for recovery.

4. Preparation of Hormones for Injection

The hormones dissolved in sesame oil were injected intramuscularily into the muscles of the thigh. A summary of the hormones used and the daily dosage of each is given in Table 1.

Table 1

The Daily Dosage of Hormones

Sex Hormone (given in 0.2 ml. of sesame oil)	Daily Dosage mg./rat
Testosterone Propionate	1.0
Oestradiol Dipropionate	0.1
Diethylstilboestrol	0.1
Progesterone	1.0
Desoxycorticosterone acetate	0.2

Testosterone was administered at a level which roughly corresponds to human dosage used in treatment of breast carcinoma. Similarily the dosage of oestradiol and diethylstilboestrol correspond to the dosage used in the treatment of carcinoma of the prostate. These amounts were determined by the ratio of the body weight to dosage. The dosage of progesterone and desoxycorticosterone were determined by a similar criterion. The human dosages used to determine the rat dosage in these two latter cases were those most commonly used in treatment of deficiencies of the two hormones.

5. Bleeding of Animals

The rats were bled by "milking" the blood from the tail into small tubes which were centrifuged within an hour after the bleeding. The sera, separated from the formed elements, were used for the determination of inorganic phosphorus and phosphatases either immediately following separation or after having been stored in the refrigerator. Sera kept in this manner for 2 or 3 days show no alteration of activity.(5). Pooling was done by mixing an equal quantity (usually 20 cmm.) of serum from each specimen. Blood specimens were obtained at approximately 10:00 A.M. unless otherwise noted.

6. Phosphatase Determinations

Serum phosphatases and serum inorganic phosphorus were determined by the method of Shinowara and Jones (38) as modified by Gould and Schwachman (13). This procedure measures the rate of hydrolysis of sodium β glycerophosphate by serum phosphatases in terms of phosphate liberated in the reaction. The phosphate liberated is measured by the method of Kuttner and Lichtenstein (33).

The unit of phosphatase as determined by this method is defined as "that amount of enzyme activity which is equivalent to one mg. phosphorus as the phosphate ion liberated during 1 hour of incubation at 37 degrees centigrade. with a substrate containing sodium β glycero-

phosphate, hydrolysis not exceeding 10% of the substrate and at an optimum pH of the reaction mixture for "acid" pH 5.0; for "alkaline" pH 9.3."

(a) Reagents

(1) Substrate - buffer mixture

I. Alkaline phosphatase substrate:

0.4240 gm. sodium diethyl barbiturate (Veronal, Merck)
0.5000 gm. sodium β glycerophosphate (Eastman, Kodak)
0.2464 gm. $MgSO_4 \cdot 7 H_2O$

dissolved in 100 ml. double distilled CO_2 free water.

II. Acid phosphatase substrate:

0.4240 gm. Veronal
0.5000 gm. sodium β glycerophosphate
0.86 ml. 5N acetic acid

dissolved in 100 ml. double distilled CO_2 free water.

Substrates are prepared weekly and a drop of chloroform added as a preservative. They are stored in the refrigerator when not in use.

(2)10% trichloroacetic acid in double distilled water.

(3) Molybdic acid

Prepared daily by adding 1 part of a 7.5% sodium molybdate solution to 1 part of cold 10 N H_2SO_4 with constant shaking.

(4) Stannous chloride

I. Stock solution - 6.0 gm. $SnCl_2$ and 10 ml. concentrated HCl. Cover with a toluene layer and store in the refrigerator.

II. Dilute solution - 0.2 ml. of stock diluted to 100 ml. with cold double distilled H_2O. Keep cold and use within 4 hours.

(5) Stock phosphorus standard solutions

I. 0.3509 gm. KH_2PO_4 made up to 1000 ml. with double distilled H_2O (79.88 μgm. of P/ml.)

II. 0.4394 gm. KH_2PO_4 made up to 1000 ml. with double distilled H_2O (100.1 μgm. of P/ml.)

(b) Method

Acid phosphatase and inorganic phosphorus was determined on 0.2 ml. of a 1:10 dilution of the serum; the alkaline enzyme was estimated on 0.2 ml. of a 1:100 dilution. Diluted sera are pipetted into three 10x75 mm. test tubes and 0.4 ml. of water is added to the serum being tested for inorganic phosphorus. The three test tubes and their contents are warmed on a water bath at 37 degrees centigrade and then 0.4 ml. of acid or alkaline buffer-substrate mixture, also previously warmed, are added at timed intervals, to their respective test tubes. The phosphatases of the sera are allowed to hydrolyze the substrate at 37 degrees centigrade for 1 hour, then 0.4 ml. of trichloroacetic acid is run into

each test tube to precipitate proteins and stop the enzyme action. The mixture is centrifuged and the resultant clear supernatant fluid is decanted. From the supernatant, 0.5 ml. is withdrawn and is placed in a cuvette tube (13x100 mm.) calibrated to give a standard reading with a known concentration of phosphorus and to it are added 0.7 ml. of 0.1 N NaOH and in quick succession 0.4 ml. of molybdic acid and 0.4 ml. of $SnCl_2$. Mixing is ensured throughout the whole procedure by the thorough shaking of the solution after the addition of each of the constituents. The intensity of blue color which develops due to the formation of reduced oxides of molybdenum (32) is read after 15 minutes in a Coleman spectrophotometer (with filter P.C. 4). A blank consisting of 1.2 ml. of water, 0.4 ml. molybdic acid and 0.4 ml. $SnCl_2$ is used to obtain 100% transmittance.

The percent transmittance obtained from the spectrophotometer is converted into concentration of phosphorus by the use of a standard graph which is prepared by plotting the values of a number of different known values of phosphorus concentration against their percent transmittance.

(c) The standard graph

In obtaining the standard graph a number of factors important in the development of color have to be first considered. Among these is the time of development

and stability of color produced. Shinowara and Jones (38) state that maximum color intensity is developed in 4 minutes and is maintained for an additional 4 minutes. Kuttner and Lichtenstein (31)(32) on whose method the standard graph is based maintain that the color reaches a maximum in 2 minutes even in the cold and is stable for at least 2 hours. Tuba (42) has shown that 15 minutes represented a more adequate time for color development and that this intensity is maintained for at least 1 hour longer. To settle this controversial question an experiment was devised to test the effect of time on the development and stability of color intensity.*

Color was developed in solutions of phosphorus in the usual manner and then readings of the intensity were noted at 6, 10, 15, 20, 30 and 50 minutes after the addition of the $SnCl_2$. The results are shown in Table 2. and indicate that the color intensity reaches a maximum in 15 to 20 minutes and remains stable for at least an additional 20 minutes.

* In this and in subsequent work on the standard graph Bureau of Standards pipettes and burettes were used to measure the phosphorus solutions. A Kimball red line 100 ml. volumetric was used for the dilutions. The double distilled water and the stock solutions of sodium molybdate and $SnCl_2$ were changed frequently.

Table 2

The Effect of Time On The Development and Stability of Color Intensity

Time in Minutes	% Transmittance Mean of 5 Tests
6	55
10	55¼
15	58¾
20	59½
30	59¾
50	59½

Other factors influencing the development of color were controlled in so far as possible by keeping the pH constant at 0.70, by carrying out the reaction at room temperature and by avoiding changes in the order and rate of addition of reagents.

The range of phosphorus under examination is approximately 1 - 15 mg./100 ml. Accordingly dilutions of the two stock standard solutions with water were worked out to include this range. The procedure was then as follows: 1 ml. of the diluted stock was made up to 1.2 ml. with water, then in quick succession 0.4 ml. of molybdic acid and 0.4 ml. of $SnCl_2$ were added with shaking. The percent transmittance was read in exactly 20 minutes. The results of a number of determinations of the percent transmittance given by different concentration of phosphorus are shown in Table 3 and Fig. 1.

Table 3

The Relationship of Concentrations of Phosphorus to the Percent Transmittance (Method of Kuttner and Lichtenstein)

Dilution of Stock	μgm. of Phosphorus /ml. of colored solution	Stock I		Stock II	
		No. of det'm.	Mean % Trans.	No. of det'm.	Mean % Trans.
1:800	.0499	5	85¼		
3:1000	.1198	10	79¾		
1:250	.2002			4	70
13:2000	.2597	10	69½		
1:125	.3196	10	63¼		
9:1000	.3596	12	61¾		
1:100	.3995	20	58½		
1:125	.4004			4	57¾
11:1000	.4395	12	53½		
1:80	.4994	10	51¼		
3:200	.5993	10	47½		
3:250	.6006			2	44
2:125	.8008			4	37¾
3:125	1.2012			3	23¼
4:125	1.6016			3	14¾

Values in Table 3 and Fig. 1 represent concentrations of phosphorus in μgm. per ml. of colored solution. In order to obtain the total amount of phosphorus in μgm. present in the 2 ml. of colored solution the result is multiplied by 2. In the determination of serum acid phosphatase and serum inorganic phosphorus the total amount of phosphorus in the final colored solution is obtained from 0.01 ml. of serum and in the serum alkaline phosphatase determination from 0.001 ml. of serum. Therefore to obtain mg. of phosphorus per 100 ml. of sera, the total phosphorus

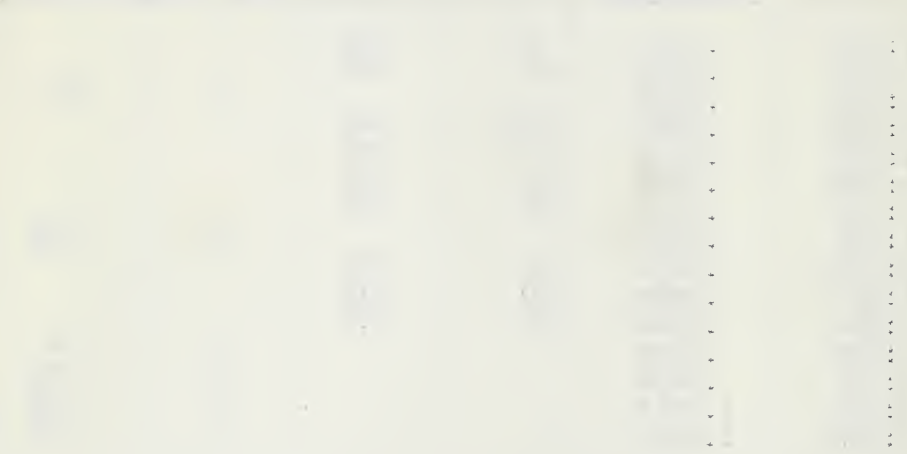
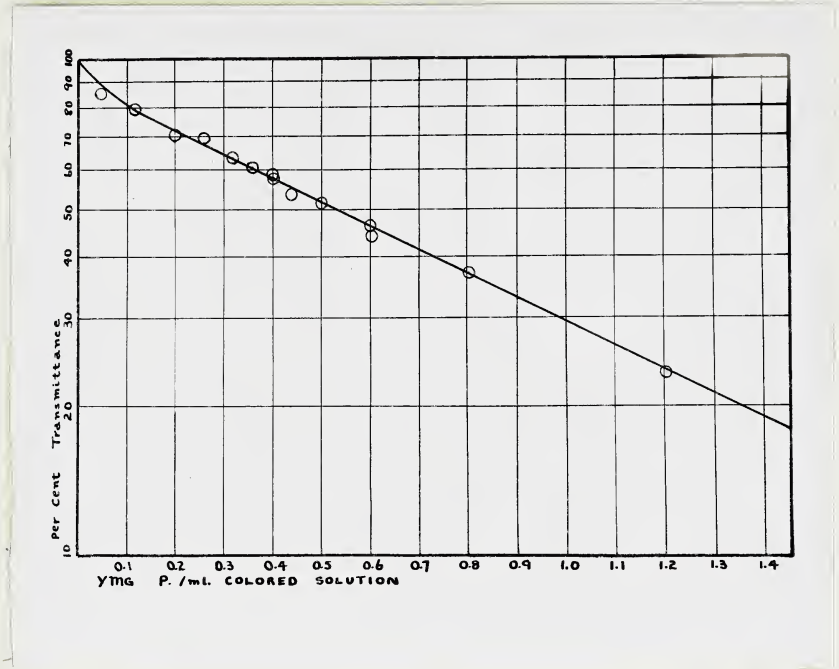


Fig. 1

The Relationship of Concentrations of Phosphorus to the Percent Transmittance (Method of Kuttner and Lichtenstein)



of the colored solution in μgm. is multiplied by 10 for the acid enzyme and inorganic phosphorus and by 100 for the alkaline enzyme.

(d) The standard error of the method

To obtain the standard error approximately 20 different estimations were done on a single concentration of phosphorus using the same apparatus and number of manipulations as were used in the estimation of serum inorganic phosphorus. The results of this experiment are shown in Table 4.

Table 4

The Standard Error of the Method (Represented by the Standard Deviation and the Coefficient of Variation)

Number of Estimations	20.0
Mean mg.P/100 ml.	9.85
Standard Deviation mg.P/100 ml.	±1.42
Coefficient of Variation	±14.0 %

In only a few instances would single determinations of phosphorus fall outside the standard deviation. The majority would be found inside the standard deviation so for practical purposes the standard deviation can be taken to represent the standard error of the method of estimating phosphorus by a single determination. A more valuable standard error of the method is given by the

coefficient of variation since it can be applied to any concentration of phosphorus.

III. EXPERIMENTAL

1. Dietary Factors Effecting the Level of Serum Phosphatases in Normal Adult Rats.

(a) Stock diets

To ascertain whether Victor fox chow or Purina fox chow caused any difference on the serum phosphatases and inorganic phosphorus when either one was fed alone, groups of 10 rats were put on each of the above diets. The animals had previously been fed on a mixture of the 2 fox chows in an amount less than they could eat. The results are seen in Table 5.

Table 5

The Effect on the Serum Phosphatases and Inorganic Phosphorus Produced by Feeding Two Different Stock Diets

Type of Fox Chow	Time After Being Put On Fox Chow	No. of Rats (pooled sera)	Serum Inorg. P mg./100ml.	Serum Acid Phosphatase Units/ 100 ml.	Serum Alk. Phosphatase Units/ 100 ml.
Victor	16 hours	10	9.1	3.5	117
	5 days	10	8.5	5.5	103
	10 days	10	6.5	2.2	113
Purina	16 hours	10	8.8	3.2	141
	5 days	10	7.8	3.3	105
	10 days	10	7.4	2.4	110

It is noted that after 16 hours on the diet the alkaline enzyme in the rats on Purina had risen to significant values above the normal mean whereas the alkaline enzyme

in the rats on Victor remained near normal levels. In accounting for these results it would seem reasonable to suppose that when underfed rats are fed Purina fox chow they overeat for the first while and later adjust themselves to a lower and more constant intake. Since, as will be shown later, the alkaline enzyme varies directly with the amount of food eaten, it follows that the activity of the enzyme will be high immediately following the feeding of an adequate diet, levelling off at lower values after the animals have adjusted themselves. Unfortunately consumption records were not kept. Why there is a difference between the levels of alkaline enzyme in rats fed on Victor and on Purina may be explained on the basis that when rats are supplied with a mixture of Victor and Purina fox chows they preferentially eat Purina.

(b) The daily variation in serum alkaline phosphatases.

During the preliminary experiments it was noted that the time of day that the rats were bled had a marked influence on the levels of alkaline phosphatase. This phenomenon was investigated by following the levels of serum enzymes and the inorganic phosphorus at intervals over a two day period.

Ten drops of blood were taken per animal at each bleeding and the resultant sera were pooled in equal quantities. This was done to minimize the effects brought

about by the loss of blood. To obtain the following results (Table 6 and Fig. 2) two groups of ten animals were used and to make the results comparable they are expressed as a percent of the 9.00 A.M. level.

Table 6

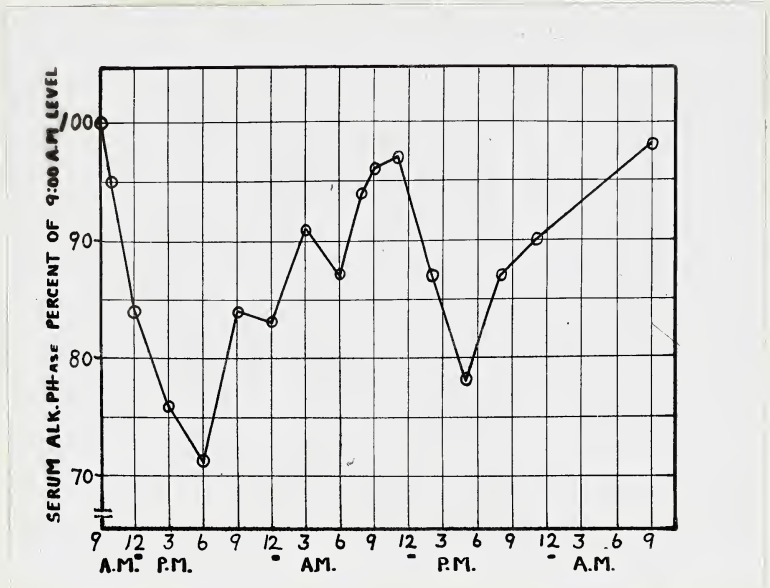
The Daily Variation in the Serum Alkaline Phosphatases in Adult Male Rats Receiving Adequate Food

No. of hrs. After Start of exp.	No. of Rats	Serum Inorg. P % of 9.00 A.M. level	Serum Acid Ph-ase % of 9.00 A.M. level	Serum Alk. Ph-ase % of 9.00 A.M. level
0(9 A.M.)	10	100	100	100
1	10	100	33	95
3	20	97	97	84
6	20	94	96	76
9	20	92	67	71
12	20	102	142	84
15	10	88	67	83
18	10	94	00	91
21	20	93	92	87
23	10	79	167	94
24	10	94	117	96
26	10	112	00	97
29	10	125	17	87
32	10	119	00	78
35	10	106	33	87
38	10	104	58	90
48	10	106	100	98

The ingestion of food by rats, which begins at their regular feeding time of 5.00 P.M. and continues throughout the night, is followed by a gradual increase in the alkaline enzyme which also continues throughout the night reaching a peak about 9.00 A.M. From 9.00 A.M. to

SLIDE 1

The Daily Variation in the Serum Alkaline Phosphatases in Adult Male Rats Receiving Adequate Food



5.00 P.M. during which time the rats are resting and consuming very little food, the alkaline enzyme falls continously.

It can be concluded from the foregoing that a rough positive correlation exists between the food intake and the level of serum alkaline phosphatase.

(c) Serum alkaline phosphatase alterations induced by lowered food intake.

To investigate further the relationship of alkaline phosphatase to the food intake (Cantor(8) an experiment was devised in which weighed amounts of food were given to groups of 6 animals and the phosphatase level was estimated at 0 hour and after 3 days on this regime. The results are seen in Table 7 and Fig. 3. No alterations were seen in the inorganic phosphorus nor the acid phosphatase.

NOTE: In Table 7 and Fig. 3 a 100% level of serum alkaline phosphatase was taken as 97 units. This is 16 units lower than the mean or actual 100% level.

The correlation coefficient calculated from the above data is equal to .97 which indicates a high degree of association between the two attributes.

Fig. 3

Scatter Diagram and Regression Lines of the Data of Table 7 (regression coefficient .97)

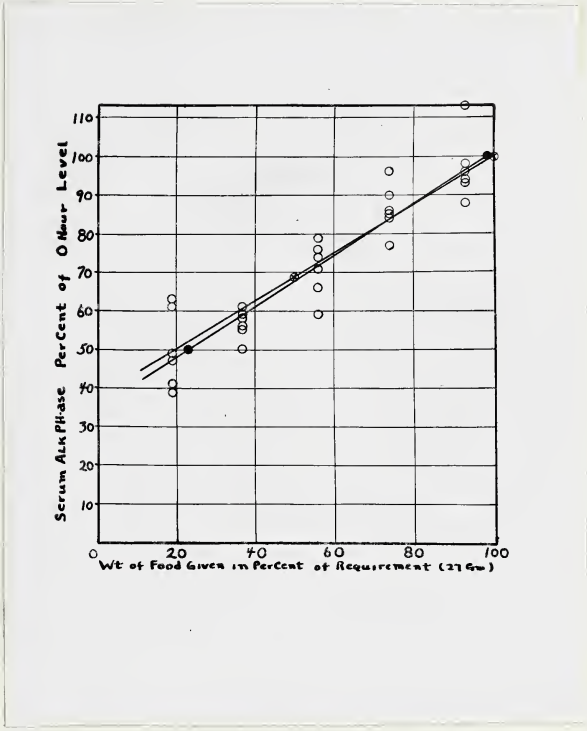


Table 7

Alkaline Phosphatase Alterations Brought About by Lowered Food Intake (30 rats)(8).

Level serum alkaline phosphatase expressed as a % of the 0 hr. value.	Weight of checkers eaten expressed in gms./day/rat					Total
	5	10	15	20	25	
106-115	—	—	—	—	1	1
96-105	—	—	—	1	2	3
86-95	—	—	—	2	3	5
76-85	—	—	2	3	—	5
66-75	—	—	3	—	—	3
56-65	2	4	1	—	—	7
46-55	2	2	—	—	—	4
36-45	2	—	—	—	—	2
Total	6	6	6	6	6	30

(d) The effect of starvation on serum phosphatases and inorganic phosphorus.

To determine how low the serum alkaline phosphatase would go with complete absence of food (Cantor (8) it was decided to starve rats until a basal level was reached. The results of starving 6 rats are shown in Table 8 and Fig. 4.

It is seen that a basal level is reached after 2 days of starvation. It is also to be noted that this level is not the one expected from the extrapolation of the graph of Fig. 3.

Fig. 4

The Effect of Starvation on Serum
Alkaline Phosphatase in 6 Rats

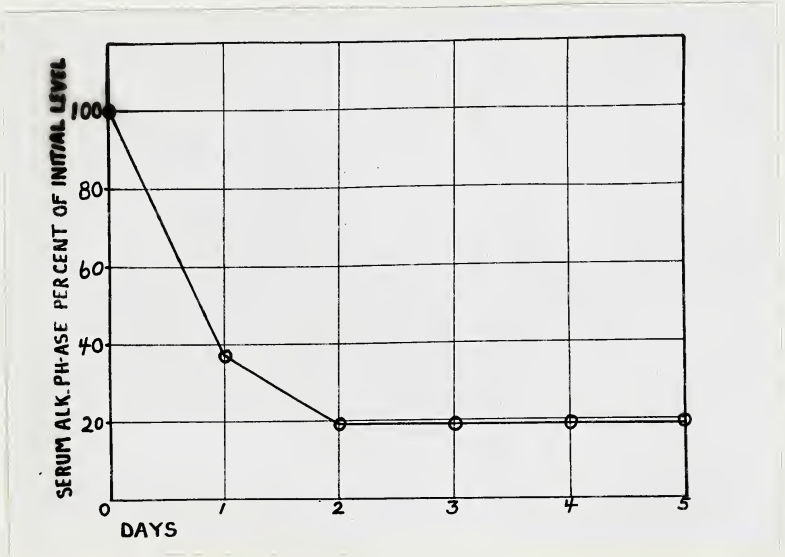


Table 8

The Effect of Starvation on Serum Phosphatases and Inorganic Phosphorus in 6 Rats (pooled sera)(8)

No. of days Starved	Serum Inorg. P mg./100ml.	Serum Acid ph-ase units/100ml.	Serum Alk. ph-ase units/100ml.	Alk. ph-ase expressed as % of initial level
0	6.8	2.1	125	100
1	7.1	1.8	46	37
2	7.5	1.8	24	19
3	7.4	2.7	24	19
4	7.1	3.0	24	19
5	6.2	2.9	24	19

(e) The relationship of the fat content of the diet to alkaline phosphatase levels.

From the foregoing experiments it became increasingly apparent that food played a major part in determining the levels of the serum alkaline enzyme. However the knowledge that food did effect the levels, did not offer any solution as to the mechanism involved. Three possibilities present themselves. (1) That the alkaline phosphatase varied directly with the intake of calories. (2) That the alkaline phosphatase varied directly with the energy expended by the animal. (3) That the alkaline phosphatase varied directly with the intake of some constituent of the food.

By an experiment devised by Cantor and Capsey

(8) the results of which are shown in Tables 9 and 10, the first two explanations were eliminated and the third was shown to be correct. It had previously been shown by Gould (14), Wiel and Russell (44) and by Capsey (9) that fat played an important part in determining the enzyme level. As a result it was decided to lower the calorie intake of the diet. while at the same time to keep the quantity of fat at levels such as that eaten by a rat getting adequate food. As can be seen by the results the alkaline phosphatase remained unaltered even with the lowered calorie intake.

Table 9a

Percent Composition of Checkers Fed as a Stock Diet

	% Composition	Cals./100gms.
Fat	7.4	66.6
Protein	30.0	120.0
CHO	58.0	232.0
Fibre	4.5	
Total	99.9	418.6

In 27 gms. of checkers there are 418.6x27/100 or 113 cals. Of this 66.6/100x27 or 18 cals. are obtained from fat. Therefore a diet was made up containing 22 cals. (approx. corresponds to the cals. contained in 5 gms. of checkers) of which 18 cals. came from fat. The composition of the diet was as follows:

Table 9B

High Fat Diet

fat	2 gm.	18 cals.
casein	1 gm.	4 cals.
CHO	0 gm.	—
yeast	½ gm.	—
Salt	½ gm.	—
	4 gms.	22 cals.

Table 10

The Relationship of the Fat Content of the Diet to Serum Alkaline Phosphatase Levels

Time in Days	Rats Receiving 22 cals. 18 of which came from fat			Rats Receiving 22 cals. Obtained from Stock Diet		
	P mg. %	Acid units %	Alk. units %	P mg. %	Acid units %	Alk. units %
0	7.6	2.3	100	7.4	2.2	95
3	7.5	4.0	101	7.6	2.2	47

(f) The daily food intake of adult rats

Since it became evident that food played such an important role in determining the level of serum alkaline phosphatase it seemed necessary that a record be kept of the amount of food consumed. To determine the amount of food ingested by rats fed ad lib. under the various experimental conditions the food was weighed for the two days prior to the bleeding and the mean of the two days divided by the number of rats was taken to represent the number of gms. eaten/rat/day. Results are seen in Table 11.

SLIDE 7

The Daily Food Intake of Adult Rats

Condition of the Experimental Animal	Hormone Injected	Am't. of Food Ingested /rat/day in gms.
Normal Male		27
Normal Female		15
Castrated Male		Decreases from 27 gms. at time of operation to 15 gms. 8 wks. later. Marked fluctuations then occur.
Castrated Female		15
Normal Males	Testosterone	23
	Oestradiol	10
	Diethylstilboestrol	10
	Progesterone	21
	Estradiol & Progesterone	10 during first week increasing to 20 in 2nd.
	Desoxycorticosterone	19
Castrated Males	Testosterone	24
	Oestradiol	10
	Diethylstilboestrol	10
	Progesterone	20
	Estradiol & Progesterone	10 during first week increasing to 20 in 2nd.
	Desoxycorticosterone	11
Normal Females	Testosterone	17
	Oestradiol	14
	Diethylstilboestrol	6
	Progesterone	15
	Desoxycorticosterone	15
Castrated Females	Testosterone	11
	Oestradiol	8
	Diethylstilboestrol	4
	Progesterone	13
	Desoxycorticosterone	13

2. Mean Values for Serum Phosphatases and Inorganic Phosphorus

(a) Normal animals

The mean, standard error of the mean, standard deviation and the range for serum inorganic phosphorus and serum acid and alkaline phosphatase in normal adult male and female albino rats is presented in Tables 12 and 13 and in Figs. 5 to 10.

SLIDE 2

The Mean, Standard Error of the Mean, Standard Deviation and The Range for Serum Inorganic Phosphorus and Serum Acid and Alkaline Phosphatase in Normal Adult Male Rats

	No. of Rats	Mean	Standard Error of Mean	Standard Deviation	Range
Serum Inorg. P.	141	8.0mg./ 100ml.	±.2 mg/ 100ml.	±1.2 mg./ 100ml.	5.4-12.4 mg./100ml.
Serum Acid Ph-ase	130	2.6 units /100ml.	±.2 units /100ml.	±1.1 units /100ml.	.3-5.2 units/100ml.
Serum Alk. Ph-ase	151	113 units /100ml.	±5 units /100ml.	± 28 units /100ml.	35-189 units/100ml.

The Mean, Standard Error of the Mean, Standard Deviation and The Range for Serum Inorganic Phosphorus and Serum Acid and Alkaline Phosphatase in Normal Adult Female Rats

	No. of Rats	Mean	Standard Error of Mean	Standard Deviation	Range
Serum Inorg. P.	120	7.2mg./ 100ml.	±.3 mg./ 100ml.	±1.5 mg./ 100ml.	4.7-15.5mg. /100ml.
Serum Acid Ph-ase	120	2.6 units /100ml.	±.2 units /100ml.	±0.9 units /100ml.	0.0-5.4 units/100ml.
Serum Alk. Ph-ase	122	70 units /100ml.	± 4 units /100ml.	± 21 units /100ml.	34-128 units/100ml.

Fig. 5

Histogram of the Distribution of Serum Inorganic Phosphorus Levels in 141 Normal Adult Male Rats

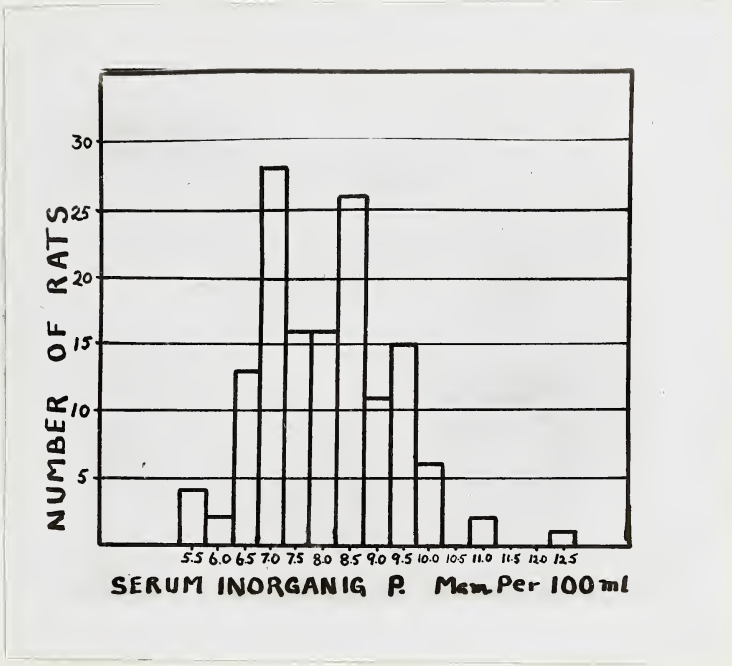


Fig. 6

Histogram of the Distribution of Serum Acid Phosphatase Levels in 130 normal Adult Male Rats

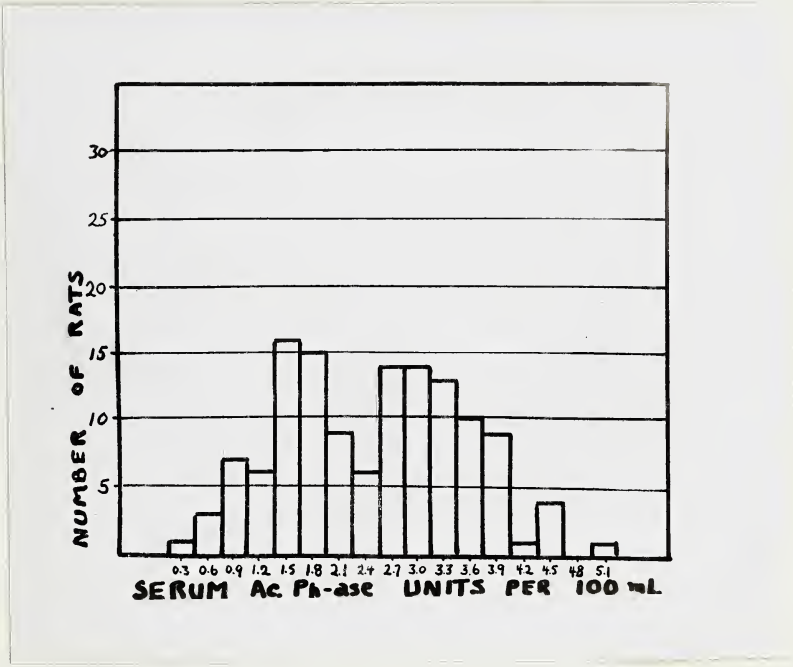


Fig. 7

Histogram of the Distribution of Serum Alkaline Phosphatase Levels in 151 Normal Adult Male Rats

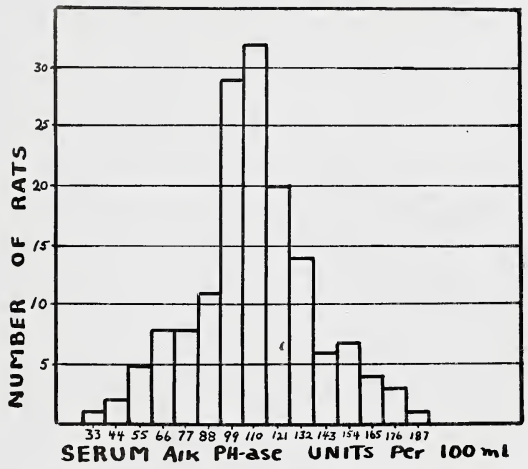


Fig. 8

Histogram of the Distribution of Serum Inorganic Phosphorus Levels in 120 Normal Adult Female Rats

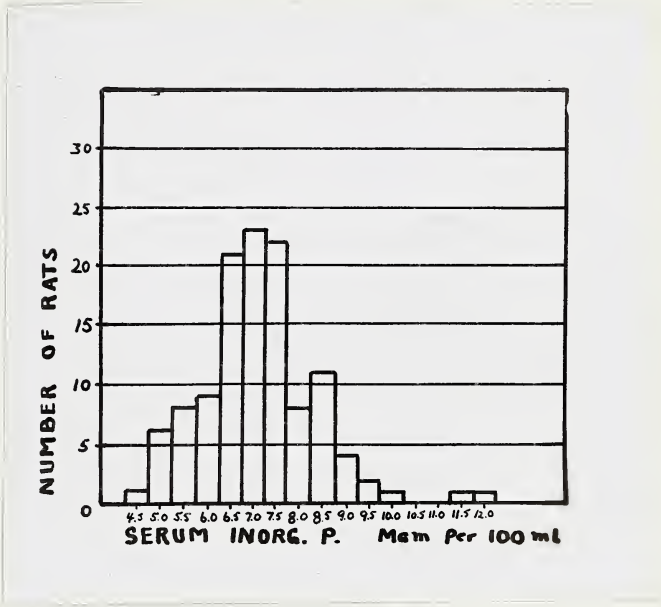


Fig. 9

Histogram of the Distribution of Serum Acid Phosphatase Levels in 120 Normal Adult Female Rats

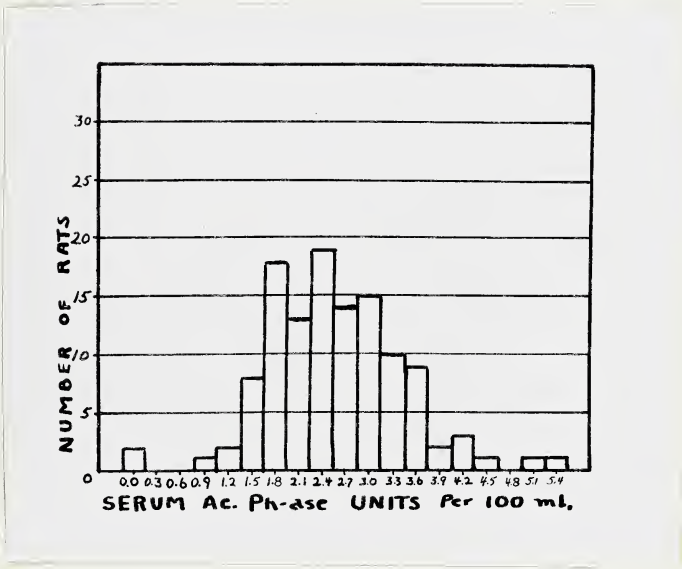
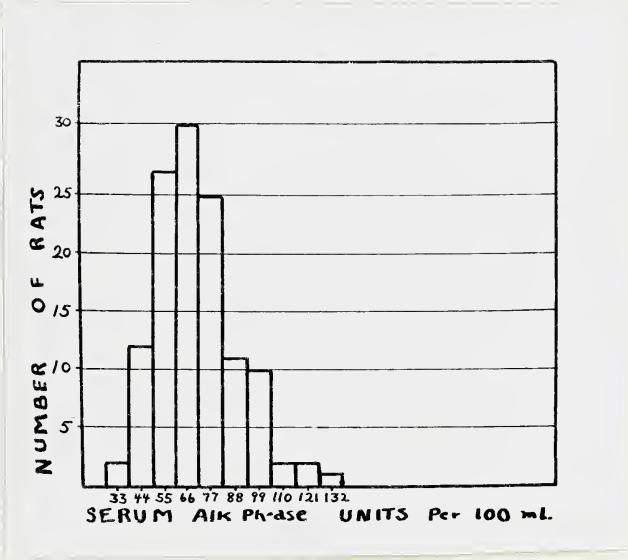


Fig. 10

Histogram of the Distribution of Serum Alkaline Phosphatase Levels in 122 Normal Adult Female Rats



(b) Castrated animals

The effects produced by castration on the normal mean of serum phosphatases and inorganic phosphorus of adult male and female rats are illustrated by Tables 14 and 15 and Figs. 11 and 12.

In these experiments the levels of enzymes in a group of 10 animals were followed at weekly intervals for 8 weeks in both sexes and every other week in the male for an additional month. A number of other castrated groups of males and females were examined for levels of phosphatase at a greater interval than 8 weeks.

SLIDE 3

The Effect of Orchidectomy on the Serum Phosphatases and Inorganic Phosphorus (mean obtained from 10 rats)

No. of Weeks After Operation	Serum Inorg. P. mg./100ml.	Serum acid ph-ase units/100ml.	Serum alk. ph-ase units/100ml.
preoperative	8.0	2.3	106
1	8.2	3.5	103
2	8.5	2.4	96
3	8.4	2.6	96
4	8.1	4.2	95
5	8.2	2.8	80
6	8.1	3.7	72
7	8.0	1.0	69
8	7.9	2.5	63
10	7.2	3.2	85
12	5.8	2.0	96

SLIDE 4

The Effect of Oophorectomy on the Serum Phosphatases and Inorganic Phosphorus (mean obtained from 10 rats)

No. of Weeks After Operation	Serum Inorg. P. mg./100ml.	Serum acid ph-ase units/100ml.	Serum alk. ph-ase units/100ml.
preoperative	6.2	3.1	69
1	6.8	2.6	61
2	6.4	2.0	63
3	5.9	1.9	57
4	5.8	3.1	90
5	5.9	5.3	86
6	5.6	5.4	77
7	6.8	1.9	80
8	6.0	3.0	70

In male rats tested beyond 12 weeks after castration the alkaline phosphatase is found to fluctuate between 65-100 units whereas the phosphorus remains at low levels.

No significant alterations occur in the female tested beyond 8 weeks.

(c) The weekly variation in serum phosphatases and inorganic phosphorus.

Phosphatase and phosphorus levels were determined in a group of 10 normal adult male rats weekly for a period of 8 weeks. Fig. 12 represents the fluctuations of the means graphically and Table 16 represents a typical example of the variations occurring in the individual rats during the 8 week interval.

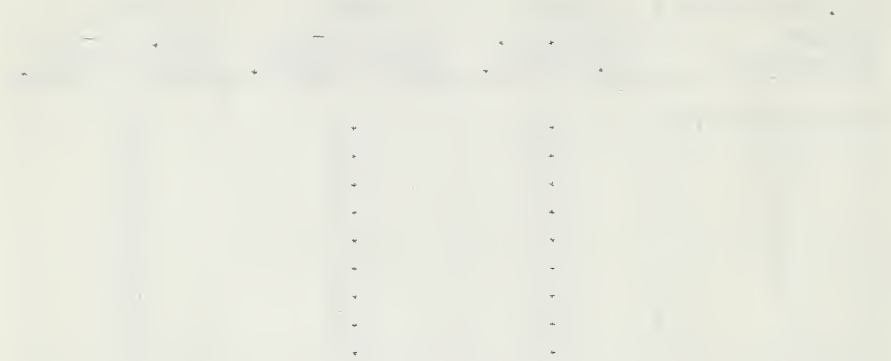
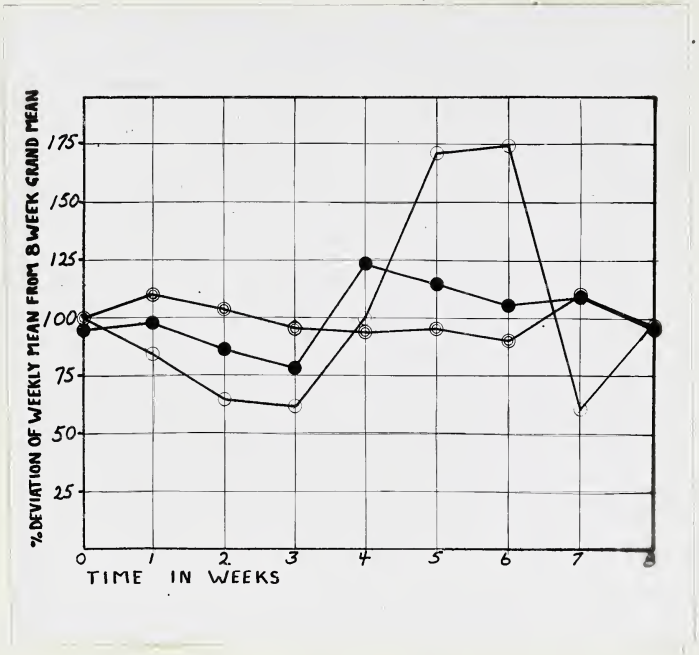


Fig. 11

The Effect of Orchidectomy on the Serum Alkaline Phosphatase
(each point on the graph corresponds to a mean obtained from 10 rats)

Fig. 12

The Weekly Variation in Serum Phosphatases and Serum Inorganic Phosphorus in Adult Rats (data from Table 15)



◎-Serum Inorganic Phosphorus
○-Serum Acid Phosphatase
●-Serum Alkaline Phosphatase

Table 16

The Weekly Variation in the Serum Phosphatase and Inorganic Phosphorus in Normal Adult Male Rats

Rat No.	Serum Inorg. P mg./100ml.		Serum Acid Ph-ase units/100ml.		Serum Alk. Ph-ase units/100ml.	
	0 hr.	8 wks.	0 hr.	8 wks.	0 hr.	8 wks.
1	6.8	6.3	3.4	1.7	110	120
2	7.8	6.8	3.9	1.7	105	108
3	6.7	7.0	4.5	2.6	117	117
4	6.1	6.7	2.2	1.9	120	125
5	7.0	7.7	4.5	1.7	135	131
6	7.1	7.4	3.1	1.9	110	114
7	7.2	7.4	3.7	1.5	114	123
8	7.1	7.2	2.6	1.7	91	119
9	6.5	7.1	3.4	1.5	82	97
10	7.1	6.7	3.4	1.6	118	115
Average Mean	6.8	7.0	3.5	1.9	110	117

For a complete table of the means from which Fig. 12 was obtained see Table 15.

These results show a greater fluctuation than do Capsey's (9) who tested the weekly variation in a group of 10 rats at weekly intervals over a period of 6 weeks.

By obtaining the grand mean of the weekly means of the data from which Fig. 12 is derived, one is able to calculate a standard deviation of the weekly means about the grand mean, and a coefficient of variation. This coefficient represents the weekly variation that can be expected in a group of 10 animals. The coefficient of

of variation for inorganic phosphorus is ±7%, for alkaline phosphatase ±14% and for acid phosphatase ±41%. For deciding whether results were significant or not higher normal weekly variations than these were used. (For alkaline phosphatase ±20%, for inorganic phosphorus ±10%, for acid phosphatase ±50%). This was done to ensure that the above rats used in obtaining the weekly variations were not exceptional in having phosphatases which did not vary a great deal.

The variation in the alkaline phosphatase and the serum inorganic phosphorus can be accounted for by the experimental error of the method (approximately ±14%) but no explanation can be offered for the huge variations which occur in the acid enzyme.

3. Serum Phosphatases and Inorganic Phosphorus in Relation to the Oestrous Cycle

It was felt that the serum phosphatases of the female rat might be affected by the oestrous cycle since here was a source of naturally fluctuating hormone levels and also since it had been shown by Atkinson (2)(3)(4) that when estrogen levels were raised the level of alkaline phosphatases in the uterus as shown by staining, was also raised. The converse also was shown to be true.

To obtain rats in the various stages of oestrous a group of 30 was bled at 12.00 P.M. This time was chosen

since rats usually come into heat at night. Vaginal smears were taken prior to bleeding and the stage of oestrous determined from them. Results are shown in Table 17.

Table 17

Serum Phosphatases and Inorganic Phosphorus in Relation to the Oestrous Cycle

No. of Rats	Stage of Oestrus	Serum Inorg. P. mg./100ml.	Serum Acid Ph-ase units/100ml.	Serum Alk. Ph-ase units/100ml.
1	I	8.3	1.6	49
9	II	6.9	2.2	56
1	III	6.5	4.5	73
4	IV	7.2	2.2	47
15	V	6.8	2.5	57

There are serious objections to this experiment. In both stage I and III there is only one rat. Taking the overall picture however there appears to be no effect produced on the serum phosphatases nor inorganic phosphorus when female rats are in the stage of heat as contrasted with those rats that are not in heat.

4. The Effect of Sex Hormones on the Serum Phosphatases and Inorganic Phosphorus.

Testosterone, oestradiol, diethylstilboestrol, progesterone, progesterone after oestradiol priming and desoxycorticosterone were injected into separate groups of castrate and intact animals. The hormones were injected singly in oily solution over a period of time and at inter-

vals noted in Tables 18 and 19. These tables together with Figs. 13 and 14 depict the results obtained.

SLIDE 5

The Effect of Sex Hormones on the Serum Phosphatases and Inorganic Phosphorus of Adult Male Rats (each result the mean of 10 rats)

Sex Hormone & Dosage	No. of days after initial inject.	No. of injections	Serum Inorg. P. mg./100 ml.		Serum Acid Ph-ase units/100ml.		Serum Alk. Ph-ase units/100ml.	
			Cast. males	Normal males	Cast. Males	Normal males	Cast. males	Normal males
Testosterone Propionate 1 mg./day/rat	0	0	5.1	8.0	1.6	2.1	70	97
	15	14	10.8	7.8	.5	4.3	62	75
	31	21	7.1	7.5	1.6	3.0	85	102
Oestradiol dipropionate 1 mg./day/rat	0	0	6.3	8.5	2.4	3.1	98 4	114
	5	4	7.0	8.5	1.9	3.2	61	76
	9	6	5.9	6.2	1.2	3.8	40	61
Diethylstilboestrol 1 mg./day/rat	0	0	6.5	8.8	1.8	2.4	58	122
	5	4	5.8	8.5	2.4	3.9	49	94
	9	6	5.6	6.8	3.3	4.0	68	82
Progesterone 1 mg./day/rat	0	0	5.7	10.1	2.4	3.1	76	115
	7	6	5.2	7.4	1.5	3.7	55	74
	14	12	8.9	6.5	1.9	4.5	66	66
Progesterone following oestradiol dipropionate 1 mg.oest./day/rat 1 mg.prog./day/rat	0	0	5.4	10.3	3.1	3.1	90	102
	7	2 oest.	4.5	8.4	1.9	2.3	42	39
	14	6 prog.	5.5	6.6	2.0	3.6	59	31
Desoxycorticosterone acetate 2 mg./day/rat	0	0	5.7	7.0	2.5	1.9	81	90
	7	6	6.5	6.2	1.4	2.4	70	82
Sesame Oil 2 ml./day/rat	0	0	5.9	8.8	2.1	2.4	83	116
	14	13	6.0	9.3			72	85

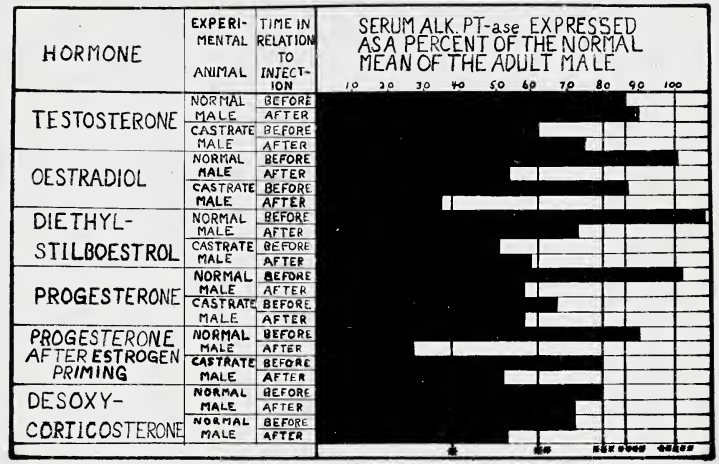
SLIDE 6

The Effect of Sex Hormones on the Serum Phosphatases and Inorganic Phosphorus of Adult Female Rats (each result the mean of 10 Rats)

Sex Hormone & Dosage	No. of days after initial inject.	No. of injections	Serum Inorg. P. mg./100 ml.		Serum Acid Ph-ase units/100 ml.		Serum Alk. Ph-ase units/100 ml.	
			Cast. Fem.	Normal Fem.	Cast. Fem.	Normal Fem.	Cast. Fem.	Normal Fem.
Testosterone propionate	0	0	4.9	7.9	2.3	2.5	78	83
1 mg./day/rat	14	13	6.5	8.9	1.9	1.6	45	76
Oestradiol Dipropionate	0	0	5.8	7.0	3.5	2.4	62	71
.1 mg./day/rat	7	6	6.8	8.6	2.2	2.1	58	63
Diethylstilb-oestrol	0	0	5.8	5.8	2.4	2.0	54	61
.1 mg./day/rat	7	6	6.3	7.3	2.6	1.8	26	43
Progesterone	0	0	6.5	7.2	5.0	3.5	76	67
1 mg./day/rat	14	13	7.0	8.5	1.4	2.7	46	64
Desoxycortico-sterone acetate	0	0	6.9	7.1	3.1	2.7	85	86
.2 mg./day/rat	7	6	7.2	7.0	2.4	2.6	47	50

Fig. 13

Serum Alkaline Phosphatase Variations in the Adult Male Rat
Resulting from the Injection of Sex Hormones



* Level below which female results expressed as a mean of 10 rats are regarded to be significantly altered from their normal mean.

** Mean of normal female rats.

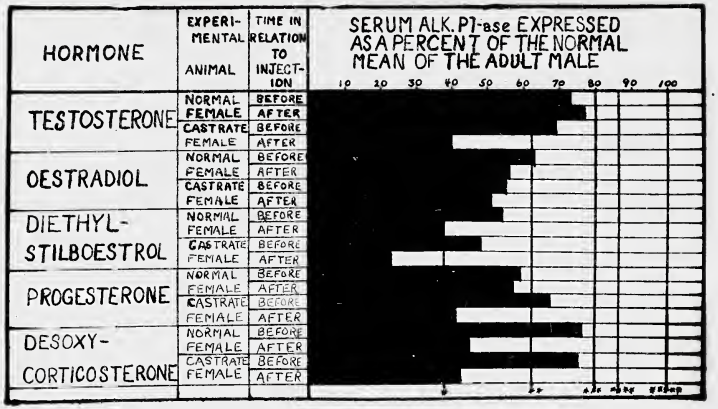
*** Level above which female results expressed as a mean of 10 rats are regarded to be significantly altered from their normal mean.

**** Level below which male results expressed as a mean of 10 rats are regarded to be significantly altered from their normal mean.

***** Mean of normal male rats.

Fig. 14

Serum Alkaline Phosphatase Variations in the Adult Female Rat
Resulting from the Injection of Sex Hormones



* Level below which female results expressed as a mean of 10 rats are regarded to be significantly altered from their normal mean.

** Mean of normal female rats.

*** Level above which female results expressed as a mean of 10 rats are regarded to be significantly altered from their normal mean.

**** Level below which male results expressed as a mean of 10 rats are regarded to be significantly altered from their normal mean.

***** Mean of normal male rats.

IV. DISCUSSION OF RESULTS

From the results of Figs. 13 and 14 and Tables 18 and 19 are noted the following:

(a) Testosterone in doses of 1 mg. per rat per day causes a significant rise in the serum inorganic phosphorus of castrated males and females and in normal females; but no alteration in normal males. Alkaline serum phosphatase activity does not vary significantly in normal or in castrate rats of either sex.

(b) Oestradiol in doses of 0.1 mg. per rat per day causes a lowering of the serum inorganic phosphorus in normal and castrated males but in the normal and castrate females it produces the opposite effect and a rise occurs. The alkaline phosphatase in castrated and normal males is markedly lowered whereas in the female no important alterations are seen.

(c) Diethylstilboestrol in daily doses of 0.1 mg. per rat causes an effect on the serum inorganic phosphorus similar to oestradiol. With regard to the alkaline phosphatase significant alterations are seen in the normal male and in the castrated and normal female. (These last two results are taken to be significant since they fall outside the limits expected of normality whereas the .0 hour values were within the range.).

(d) Progesterone when administered in 1 mg. doses caused a rise of the levels of serum inorganic phosphorus in castrated males and in normal females and a lowering in normal males. Castrated females were not affected. A lowering in the alkaline phosphatase occurred in the normal male rats only, there being no evident response in the other animals.

(e) When progesterone was administered after estrogen priming the same results were obtained in the adult male as with progesterone alone. Unfortunately the effect in the female was not completed due to an error in the experiment.

(f) Desoxycorticosterone in a dose of 0.2 mg. /rat/day produced no significant alterations in serum inorganic phosphorus or in serum alkaline phosphatase.

Difficulty was experienced in the determination of acid phosphatases since they are found to alter quite markedly on standing or with hemolysis. As a result no conclusions can be made about their variations.

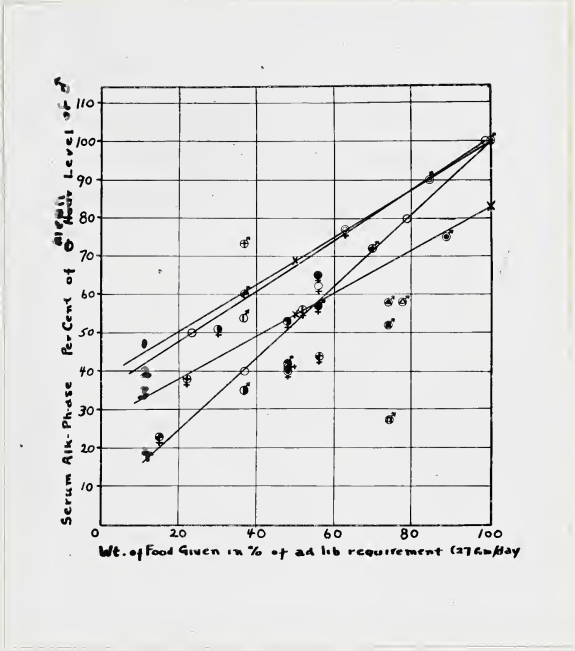
To see whether the fall in serum alkaline phosphatase which is noted with the castration of male animals and with the injection of some of the sex hormones is due to a deficiency of food, a scatter diagram (Fig. 15) was made correlating the food intake expressed as a percent of the amount eaten ad lib (27 gms./rat/day) with the level of

the alkaline phosphatase expressed as a percent of the mean of the normal adult male rat (113 units/100 ml.). In addition, the regression coefficient of the above data was calculated and found to be 0.70. The correlation found here is less than that found by using the data of Cantor and Capsey (8) (Fig. 16, Table 10). However it is still great enough to indicate that all the variations occurring in serum alkaline phosphatase due to castration or injection of sex hormones can be accounted for by an altered food intake. There is one notable exception to this conclusion. This is afforded by the lowering in the enzyme which results from the injection of progesterone following priming by estrogen in normal adult males.

The lowering of phosphorus which occurs following castration in male rats and after the injection of oestradiol or diethylstilboestrol in castrated and normal males may be accounted for by the general fall in metabolism which follows these procedures. Woodard (45) found similar results in the serum inorganic phosphorus in patients receiving stilboestrol treatment for carcinoma of the prostate. He also found that this change was not associated with any changes in serum calcium. The rise in serum inorganic phosphorus following testosterone administration may be accounted for by the converse of the cause of the fall; that is, a rise in general metabolism. It has been

Fig. 15

Scatter Diagram and the Regression Lines of the Data Obtained From Fig. 13 and 14 and Table 11



1&2 -regression lines obtained from Fig.3
3&4 -regression lines obtained from the data of Fig. 13 & 14 and Table 11 - coefficient of regression 0.70

Symbols used in Fig. 15

↗ &+ attached to the symbol indicates male & female rats respectively.

○-normal
●-castrate
◎-testosterone normal
◉-testosterone castrate
◐-eostradiol normal
◑-eastradiol castrate
⊕-diethylstilboestrol normal
⊕-diethylstilboestrol castrate
△-progesterone normal
▲-progesterone castrate
◫-progesterone after estrogen normal
▣-progesterone after estrogen castrate
⊕-desoxycorticosterone normal
⊕-desoxycorticosterone castrate
X-points on regression line
○-points on regression line

suggested that this rise in serum inorganic phosphorus may be one of the mechanisms responsible for the regression of the bone metastasis in carcinoma of the breast.

In a final examination of the results of the serum alkaline phosphatase it may be well to mention again the work of Buchwald etal (6)(7) and to compare their results with the ones obtained in this experiment. Buchwald obtained an increase with testosterone in the intact and castrated female and no alteration with diethylstilboestrol in the intact and castrated male. The results presented here do not agree with these since testosterone produced no effect, whereas diethylstilboestrol produced a lowering. Kochakian (28)(29)(30) in studying the effect of sex hormones on the alkaline phosphatase, used the enzymes of the kidney and liver as his basis for comparison. He found that when male hormones were injected into castrated mice, the alkaline phosphatase level of the kidney decreased in relation to the increase in size of the kidney. The opposite occurred in rats.

Taking the overall picture, the main conclusion that can be drawn is that it is the action on the general body metabolism produced by the sex hormones which determines whether the serum alkaline phosphatase is to be altered or not. If the hormones lower the general metabolism and thus the food intake, tney lower the serum alkaline phos-

phatase activity. If they raise the metabolism and cause increased food intake they increase the alkaline enzyme activity. If they produce no marked alteration in body metabolism as shown by the level of the food intake they cause no alteration in the alkaline enzyme. A special note should be made of the level of serum alkaline phosphatase in normal adult males following the injection of progesterone after oestradiol priming. All the fall here cannot be attributed to lack of food. Further it may be concluded that fat is the constituent of the food which is responsible for the greater part of the enzyme activity exhibited in rats sera. No explanation is offered as to how or where it acts.

It may be well to remember, as Miller (36) mentions, that the literature on experimental carcinogenesis contains numerous references to decreased enzyme activities, with or without tumours (16). In many instances no reference is made to weight loss or to the amount of food consumed. In these instances reduction of the enzyme activity and its alleged significance for the origin of or relation to the neoplastic changes observed must be re-examined since similar changes may be found to be the result of simple inanition.

V. SUMMARY

1. Normal means and the weekly variations for serum inorganic phosphorus and serum acid and alkaline phosphatases are given for normal adult male and female rats.

2. Some nutritional aspects are studied concerning levels of serum alkaline phosphatase and it is concluded that the alkaline enzyme varies directly with the intake of food over the range studied and further that the fat content of the food is the factor which is responsible for the greater part of the variation.

3. Castrated male rats were studied for 12 weeks and it is concluded that the serum inorganic phosphorus fell significantly toward the end of this period. The serum alkaline phosphatase fell within 8 weeks and its alteration could be accounted for by the lowered food intake.

4. Castrated female rats were studied for 8 weeks and no alterations are seen which can be attributed to castration.

5. Sex hormone injected, castrated and intact adult male and female rats, were studied and it was shown that: (a) testosterone in both the intact and castrated male and female caused a rise in the serum inorganic phosphorus.(b) oestradiol and diethylstilboestrol in the

intact and castrated male, produced a fall in the serum inorganic phosphorus whereas in the females a rise. (c) progesterone alone or after oestradiol priming effected a rise in the serum inorganic phosphorus of the castrated male and a fall in the normal male. No alteration in the castrated female and a rise in the normal female. (d) desoxycorticosterone acetate did not alter the serum inorganic phosphorus nor serum phosphatases.

Serum alkaline phosphatase alterations caused by the injection of sex hormones can be accounted for by alterations in the food intake of the animals which occur following their injection. Thus those hormones, (estradiol in the intact and castrated male, diethylstilboestrol and progesterone in the intact male and diethylstilboestrol in the intact and castrated female) which cause a lowered food intake cause a lowering of the serum alkaline phosphatase.

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VII. ADDENDUM

Studies on the hemoglobin, hematocrit, serum protein, serum inorganic phosphorus and serum phosphatase levels in rats in relation to deficiencies of members of the vitamin B. complex.

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I. INTRODUCTION

A conjoint study was made on (a) the relationship of serum phosphatases to vitamin B complex deficiencies and (b) the relationship of anemia to vitamin B complex deficiencies.

(a) The relationship of vitamin B complex deficiencies to serum phosphatase levels.

In studying the effect of nutritional requirements on the phosphatases of yeast, Tuba (21) was able to show that thiamine caused an inhibition of the activity of the alkaline enzyme. A number of years later this inhibition of alkaline phosphatase by thiamine was shown to occur in the serum of mammals after the subcutaneous injection of the vitamin. It was suggested that vitamin B1 was probably an activator of the phosphorylation process (14). Thiamine is now shown to act as the coenzyme, cocarboxylase, which is the pyrophosphate of thiamine (9). Thus it can be seen that thiamine appears intimately concerned with phosphorus metabolism and it is quite conceivable that alterations in alkaline phosphatase would occur with a deficiency of the vitamin.

Since riboflavin, (esterified with phosphorus in the formation of Warburg's yellow enzymes,) nicotinic acid amide (present with phosphorus in coenzyme I and II) inositol (lipsitol of Wooley) and choline (tied up with phosphorus in lechthin (9)) besides thiamine are shown to

be connected to phosphorus it was decided to investigate the relationship of a deficiency of the above vitamins to the phosphatase levels. It was felt that any alteration that might reflect a change in the phosphorus level would reflect a change in the serum alkaline phosphatase.

(b) The relationship of anemia to the deficiencies of the vitamin B complex.

For the past few years much has been written concerning the various types of anemias in experimental animals produced by the deficiencies of one or all of the members of the vitamin B complex. Thus Castle (4) indicates that with complete vitamin B deficiency pernicious anemia will develop in animals. Kornberg et al (12) with riboflavin deficient rats produced granulo cytopenia and anemia. Sebrell (20) using the dog and Waisman (22) the monkey, produced hypochromic anemias on a riboflavin deficient diet. Handler (8) has related anemia to nicotinic acid amide deficiency and as well as getting a profound macrocytic anemia he found a lowering of plasma proteins. Pyridoxine deficient cattle, swine and dogs develop a microcytic hypochromic anemia and poikliocytosis (3)(16)(17). Carter et al (2) produced a rapid fall in hemoglobin and red blood cells in 60% of their rats on a pantothenic deficient diet. Inositol as the phosphoric ester has been shown to be present in nucleated red blood cells (18). Folic acid too has been shown to be factor since absence

of it in chicks leads to an anemia (10). In investigating anemia in the rat the prime purpose was to obtain concise data relating hemoglobin and hematocrit levels to various of the deficiencies of the members of the vitamin B complex. Since it was noted that a complete study on the relationship of serum protein to vitamin B complex deficiencies was lacking, this problem was undertaken along with the studies on phosphatases and anemia.

II. METHODS

1. The Experimental Animal

Young Albino (Wistar strain) rats at the end of the 3rd week were weaned and placed on their respective diets in groups of 5 females and 5 males. The animals were housed in all metal cages and given tap water ad lib.

2. The Diet

Weanling rats used as normal controls, were fed a mixture of Purina and Victor fox chow. All rats in the deficient group were fed a basic vitamin B complex free diet (J. Wyeth & Co.) and were supplemented where needed with the crystalline vitamins dissolved in a water solution. Details of the diet and supplement are as follows:

Table 1

Basic Vitamin B Free Diet

	% Composition
Sucrose	68
Vitamin free casein	18
Vegetable oil	8
U.S.P. salt mixture No. 2	4
Cod Liver Oil	2

0.1 ml./day of the above supplement was fed by forcing a pipette into the pharynx of the rat and allowing the desired quantity to run in.

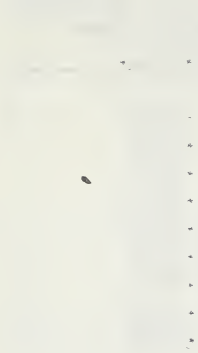
Table 2

Vitamin Supplement
(made up in 50 ml. quantities with the deficient factor left out in each case)

Vitamin	Conc. of vitamin in supplement in mg./ml.
Thiamine	.121
Riboflavin	.200
Pyridoxine	.100
Ca pantothenate	.500
Niacin	1.196
Choline	70.000
Inositol	5.357
Para Amino Benzoic Acid	2.143
Biotin	.030

3. Bleeding of Animals

Blood, obtained by tail bleeding (approx. 10 drops/rat) was centrifuged after 15 minutes and the resultant sera were pooled in equal quantities for the estimation of serum proteins and serum phosphatases. In addition 20 cmm. of blood obtained in a Sahli pipette was thoroughly mixed with 0.1% Na_2Co_3 for the estimation of hemoglobin. Blood for estimating the hematocrit level was obtained by passing the blood directly from the tail into a test tube previously dusted with potassium oxalate. Hemoglobin and hematocrit estimation were done immediately following the bleeding. The serum protein and serum phosphatase were usually estimated the same day but if not,



the sera were stored in the refrigerator.

4. The Estimation of:

(a) Serum Phosphatases

These were estimated in the same manner as is indicated in the main body of the thesis.

(b) Hemoglobin

Hemoglobin was estimated according to the method of Sanford, Sheard & Osterberg (19) using an Evelyn colorimeter with a 540 mμ filter.

(c) Hematocrits

The relative volume of packed red cells was estimated by centrifuging thoroughly shaken oxalated blood in a Van Allen hematocrit tube at 3000 r.p.m. for 30 minutes. A reading of the level of red blood cells expressed as a percent of the total blood volume was taken and the tube was centrifuged for an additional 10 minutes or until the level remained constant.

(d) Serum Protein

The serum protein was estimated by the falling drop method of Barbour and Hamilton (1) as modified by Guthrie (7).

III. EXPERIMENTAL

1. The Determination of Serum Inorganic Phosphorus and Phosphatases, Weight, Hemoglobin, Packed Red Cells and Serum Protein.

(a) During normal development of male and female rats.

Results of the above relationships are seen in Tables 3 to 7 and Figs. 1 and 2.

Table 3

Serum Phosphatases and Inorganic Phosphorus During Normal Development of Male and Female Rats

App. age of rats in wks.	Serum Inorganic P		Serum Acid Phosphatase		Serum Alk. Phosphatase	
	No. of rats	mg./100 ml.	No. of rats	units/100 ml.	No. of rats	units/100 ml.
4	5	9.4	5	3.8	5	88
6	9	9.9	9	2.0	9	94
7	14	9.4	—	—	14	99
8	25	6.6	9	2.7	29	78
9	23	6.9	13	2.9	8	105
10	2	9.8	2	1.6	2	117
11	4	8.6	4	1.0	4	88
12	2	7.2	2	2.0	2	114
13	4	6.4	4	2.4	4	89
15	2	8.2	2	2.2	2	85
18	2	5.4	—	—	2	56

Table 4

The Weight During Normal Development
Of Male and Female Rats

Mean Weight From 20 Males & 20 Females

Approx. age in weeks	Weight of the Rats Mean in gms.	% of the initial weight
3	45	100
4	80	178
5	99	220
6	123	274
7	145	323
8	153	340
9	176	390
10	190	422
11	219	487
12	239	530
13	258	574
14	261	580
15	264	586
16	267	594
18	282	626

Table 5

Hemoglobin concentration During
Normal Development of Male and
Female Rats

App. Age of rats in wks.	♀ Rats		♂ Rats	
	No. of rats	Hb. Mean in gms.%	No. of Rats	Hb. Mean in gms.%
4	8	11.3	7	10.9
6	4	13.8	5	13.3
7	3	14.6	2	14.0
8	5	15.2	4	14.7
9	6	14.9	7	14.5
10	—	—	1	14.6
12	—	—	1	15.3
13	2	14.8	2	15.3
15	2	15,0	2	15.5
18	1	14.8	1	15.0
21	1	15.2	1	15.3
23	1	15.3	1	15.4

Table 6

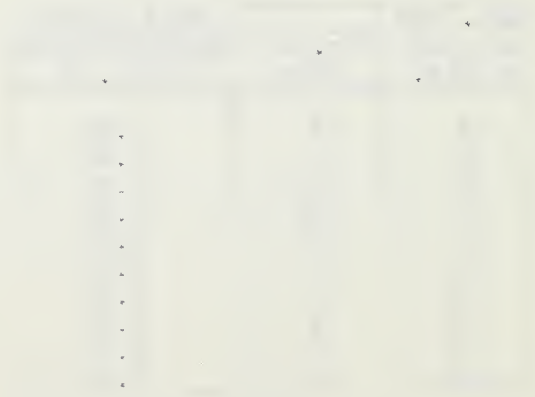
The Relative Volume of Packed Red Cells During Normal Development of Male and Female Rats.

App. age of rats in wks.	♀ Rats		♂ Rats	
	No. of rats	% R.B.C.	No. of rats	% R.B.C.
4	1	25	1	35
6	2	37	2	39
7	1	34	1	41
8	2	42	2	39
9	2	39	2	39
10	2	41	2	44
12	-	-	1	38
13	-	-	1	56
15	2	41	2	44
18	-	-	1	39

Table 7

Serum Protein in Male and Female Rats During Normal Development

App. age of rats in wks.	♂+♀ Rats	
	No, of rats	Serum Protein in gms. %
4	5	6.02
6	9	6.96
7	14	6.87
8	25	6.46
9	23	6.92
10	2	7.32
12	2	8.01
13	4	7.87
15	2	8.25
18	2	7.42



1. The Determination of Serum Inorganic Phosphorus, Phosphatases, Weight, Hemoglobin, Packed Red Cells and Serum Protein.

(b) In B Complex Deficiencies

(1) Complete Vitamin B complex
(2) Thiamine hydrochloride
(3) Riboflavin
(4) Niacin
(5) Pyridoxine
(6) Calcium pantothenate
(7) Choline
(8) Inositol

Tables 8 to 17 and Figs. 1 and 2 show the above relations.

Table 8

Serum Inorganic Phosphorus and Phosphatases in B Complex Deficiencies

Approx. age in weeks	Type of Deficiency															
	Complete Vitamin B complex				Thiamine				Riboflavin				Niacin			
	*1	**2	***3	****4	1	2	3	4	1	2	3	4	1	2	3	4
4	–	–	–	–	4	8.1	2.6	122	–	–	–	–	–	–	–	–
5	8	8.0	2.6	78	–	–	–	–	4	8.5	2.5	62	5	8.3	2.2	56
6	4	9.3	3.2	72	4	8.8	2.6	69	5	7.7	2.7	38	–	–	–	–
7	26	7.5	1.8	40	2	9.5	3.0	84	3	8.3	2.2	77	4	7.9	2.1	129
8	28	7.0	3.0	23	7	7.5	1.5	39	3	9.3	2.1	38	–	–	–	–
9	–	dead–		–	4	8.0	3.7	14	3	9.3	3.0	98	4	6.4	3.8	99
10	–	–	–	–	–	dead–		–	2	9.5	5.8	106	2	6.8	3.9	59
11	–	–	–	–	–	–	–	–	2	8.2	6.0	33	4	9.3	2.7	178
12	–	–	–	–	–	–	–	–	1	8.7	2.2	43	4	8.0	1.2	140
13	–	–	–	–	–	–	–	–	1	9.0	1.5	74	2	7.6	.8	134
14	–	–	–	–	–	–	–	–	1	8.0	6.0	60	–	–	–	–
15	–	–	–	–	–	–	–	–	1	8.7	3.3	10	4	7.5	2.3	96
16	–	–	–	–	–	–	–	–	1	7.6	2.2	26	3	7.8	3.0	43
17	–	–	–	–	–	–	–	–	–	–	dead	–	4	8.3	2.2	42
18	–	–	–	–	–	–	–	–	–	–	–	–	3	8.2	2.1	22

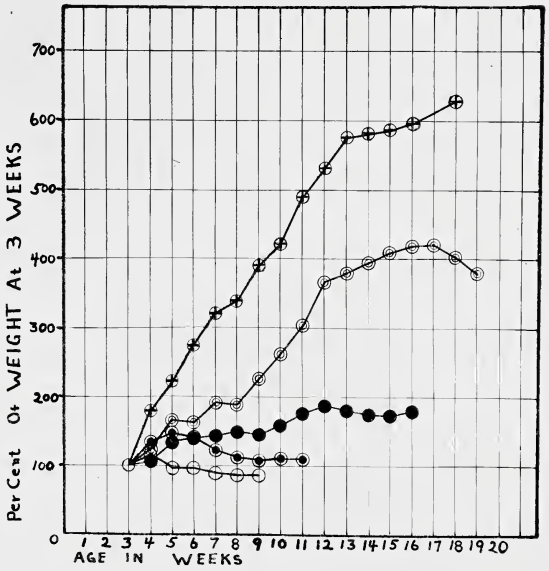
* column 1 - no. of rats
** column 2 - serum inorganic phosphorus mg./100ml. sera
*** column 3 - serum acid phosphatase units/100ml. sera
**** column 4 - serum alkaline phosphatase units/100ml. sera

Table 8 Cont'd

Approx. age in weeks	Type of Deficiency															
	Pyridoxine				Calcium Pantothenate				Choline				Inositol			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
5	5	8.0	3.2	50	4	8.0	2.5	68	—	—	—	—	—	—	—	—
6	—	—	—	—	—	—	—	—	5	9.3	3.6	74	5	9.3	3.2	58
7	3	9.2	3.2	78	—	—	—	—	—	—	—	—	—	—	—	—
8	—	—	—	—	3	8.8	5.0	127	5	9.1	2.4	69	5	9.6	3.1	61
9	4	8.8	3.0	84	—	—	—	—	5	9.3	6.2	84	4	8.8	2.3	50
10	2	7.6	2.1	75	4	9.1	—	78	—	—	—	—	—	—	—	—
11	2	7.7	3.0	99	2	8.7	3.6	94	—	—	—	—	—	—	—	—
12	4	8.0	1.9	96	2	5.7	3.7	130	2	8.8	2.0	74	2	7.0	2.1	82
13	2	8.3	1.9	92	3	6.6	3.2	90	4	7.7	3.2	50	4	8.8	3.5	78
14	—	—	—	—	2	8.2	4.0	90	4	6.2	—	98	3	9.0	—	104
15	4	6.9	4.0	52	2	7.9	3.2	145	2	8.0	2.2	66	2	8.5	3.2	78
16	4	8.2	5.6	27	2	8.0	3.2	128	—	—	—	—	—	—	—	—
17	4	7.9	3.2	40	2	8.2	2.1	96	4	7.8	2.1	36	4	8.1	3.2	54
18	4	7.5	2.2	15	—	dead	—	—	4	8.1	2.0	41	4	8.3	2.2	12
19	—	dead	—	—	—	—	—	—	4	7.2	1.9	64	4	7.5	3.1	48
20	—	—	—	—	—	—	—	—	4	7.2	3.1	10	4	6.8	3.1	18

Fig. 1

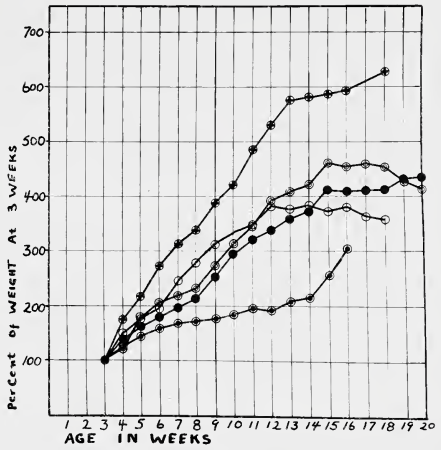
Weight During Normal Development and in B Complex Deficiencies
(Each point on the graph represents the mean of the weights of approx. 5 male and 5 female rats)



⊕ - 1. Normal male and female rats
◯ - 2. Complete vitamin B complex deficient rats
◎ - 3. Thiamine deficient rats
● - 4. Riboflavin deficient rats
◉ - 5. Niacin deficient rats

Fig. 2

Weight During Normal Development and in B Complex Deficiencies
(Each point on the graph represents the mean of the weights of approx. 5 male and 5 female rats)



⊕-1. Normal male and female rats
○-2. Pyridoxine deficient rats
◉-3. Ca pantothenate deficient rats
●-4. Choline deficient rats
◎-5. Inositol deficient rats

Table 9

Hemoglobin and Hematocrit levels in Complete Vitamin B Complex Deficient Male and Female Rats (Hb in gms./100 ml. of blood (black type) relative volume of packed red cells - hematocrit expressed as a percent (red type).

	Rat No.	Age of Rat in Weeks				
		5	6	7	8	9
♀	1	14.343	—	—	14.845	dead
	2	—	14.858	dead	—	—
	3	14.0	—	—	15.637	dead
	4	—	14.5	—	—	13.942
	5	—	—	14.336	dead	—
♂	6	—	—	13.137	dead	—
	7	14.346	—	—	14.3	dead
	8	—	13.751	dead	—	—
	9	13.1	—	—	12.947	10.645
	10	14.8	dead	—	—	—

Table 10

Hemoglobin and Hematocrit levels in Thiamine Deficient Male and Female Rats (HB in gms./100ml. of blood (black type) relation volume of packed red cells expressed as a percent (red type)

	Rat No.	Age of Rat in Weeks			
		5	7	8	9
♀	1	12.224	dead	—	—
	2	—	14.633	—	15.133
	3	13.2	dead	—	—
	4	—	15.6	dead	—
	5	—	—	14.739	14.344
♂	6	—	—	16.048	dead
	7	12.730	—	—	14.342
	8	—	14.141	—	14.736
	9	13.1	dead	—	—
	10	—	—	14.641	dead

Table 11

Hemoglobin and Hematocrit Level in Riboflavin deficient Male and Female Rats (Hb. in gms./100ml. of blood (black type) relative volume of packed red cells expressed as a percent (red type)

	Rat No.	Age of Rat in Weeks											
		5	6	7	8	9	10	11	12	13	14	15	16
♀	1	13.7 38	15.2	dead	—	—	—	—	—	—	—	—	—
	2	—	14.3 26	—	13.9	14.8	14.4 33	—	—	14.8 30	—	—	14.2 41
	3	13.8	—	14.3	—	13.7	14.5	14.3 36	—	—	14.3 43	—	—
	4	—	14.3	v —	14.4	dead	—	—	—	—	—	—	—
	5	13.7	dead	—	—	—	—	—	—	—	—	—	—
♂	6	—	14.6 46	dead	—	—	—	—	—	—	—	—	—
	7	13.4 35	—	15.0 51	dead	—	—	—	—	—	—	—	—
	8	—	15.6	dead	—	—	—	—	—	—	—	—	—
	9	—	14.8	—	14.6 38	14.2	15.2 38	—	14.6 47	—	—	14.3 47	—

Table 12

Hemoglobin and Hematocrit Level in Niacin deficient Male and Female Rats (Hb. in gms./100ml. of blood (black type) relative volume of packed red cells expressed as a percent (red type)

	Rat No.	Age of Rat in Weeks											
		5	6	7	9	10	11	12	13	15	16	17	18
♀	1	12.6 37	—	13.8	—	—	13.5 35	—	—	—	13.2 28	—	—
	2	—	14.2	—	14.8 39	—	—	13.7	dead	—	—	—	—
	3	9.0	— v	13.8 43	—	—	13.5	—	—	13.2	—	—	—
	4	—	14.3	—	14.5	—	—	13.7 38	—	14.2 35	—	13.9 30	—
	5	11.5	—	—	—	13.7 38	—	—	12.2 36	—	—	14.4 36	—
♂	6	—	14.3	—	—	13.9 34	—	—	12.7 34	—	—	13.9 31	—
	7	10.0 43	—	14 1.0	—	—	13.6 42	—	—	14.6 43	—	13.9 36	—
	8	—	14.7	—	14.0 33	—	—	13.4	—	12.2	—	—	—
	9	12.7	—	14.8 42	15.2	—	13.9	—	—	—	14.4	—	15.4
	10	—	14.1	—	13.4	—	—	13.2 31	—	—	13.2 30	—	—

Table 13

Hemoglobin and Hematocrit levels in Pyridoxine Deficient Male and Female Rats (Hb in gms./100ml. of blood (black type) relative volume of packed red cells expressed as a percent (red).

	Rat no.	Age of Rat in Weeks											
		5	6	7	9	10	11	12	13	15	16	17	18
♀	1	11.434	dead	—	—	—	—	—	—	—	—	—	—
	2	—	14.4	—	14.640	—	—	15.2	—	15.1	—	13.939	—
	3	10.1	—	14.733	—	—	15.745	—	—	13.4	—	14.542	—
	4	—	14.3	14.3	—	—	—	15.439	—	—	14.8	—	15.1
	5	10.3	—	14.3	—	13.143	—	—	13.941	—	13.933	—	13.8
♂	6	—	13.5	—	—	—	13.733	14.339	—	14.840	—	—	14.4
	7	10.837	—	14.036	—	—	13.940	—	—	14.4	—	13.936	—
	8	—	14.3	—	14.139	—	—	14.8	—	14.638	—	14.540	—
	9	—	13.5	—	14.5	—	—	14.841	—	—	14.6	—	14.4

Table 14

Hemoglobin and Hematocrit Levels in Calcium Pantothenate Deficient Male and Female Rats (Hb in gms./100ml. of blood (black type) relative Volume of Packed Red cells expressed as a percent (red).

	Rat No.	Age of Rat in Weeks										
		5	7	8	10	11	12	13	14	15	16	17
♀	1	12.729	—	14.2	—	—	14.0 48	—	—	14.738	—	13.7 35
	2	—	14.845	—	15.236	—	—	15.3	—	—	15.0	—
	3	14.4	—	15.337	—	13.832	—	—	—	14.134	—	—
	4	—	14.8	—	15.2	—	—	14.6 50	—	—	15.352	—
♂	5	13.7	—	—	—	13.837	—	—	14.334	—	—	—
	6	14.337	dead	—	—	—	—	—	—	—	—	—
	7	—	14.642	dead	—	—	—	—	—	—	—	—
	8	14.3	—	14.936	—	—	—	15.247	—	—	—	—
	9	—	13.9	—	14.3 39	—	15.0 55	—	15.6	—	—	14.648

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Table 15

Hemoglobin and Hematocrit Levels in Choline deficient Male and Female Rats (Hb in gms./100ml. of blood (black type) relative volume of packed red cells expressed as a percent (red).

	Rat No.	Age of Rat in Weeks											
		6	8	9	12	13	14	15	17	18	19	20	23
♀	1	14.6 36	—	14.3	—	—	13.5 47	—	—	13.8 28	—	14.8	15.3
	2	—	14.7 40	—	—	12.7	—	—	14.6	—	14.1 36	—	13.4
	3	14.3	—	14.8 38	—	—	14.1	dead	—	—	—	—	—
	4	—	15.1	—	—	14.6 33	—	—	13.9 31	—	14.3 47	—	13.9
	5	13.7	—	14.4	13.6 22	—	—	14.4 41	—	13.9	—	14.7	13.2
♂	6	—	14.6	—	14.4 36	—	—	15.2 40	—	14.7 40	—	13.9	13.4
	7	13.4 40	—	14.1	—	—	13.4 52	—	—	—	13.2 43	—	13.7
	8	—	15.3 44	—	—	14.0	—	—	14.3 30	—	13.9 39	—	12.6
	9	16.6	—	14.6 34	—	—	12.8	—	—	12.9 29	—	—	14.8
	10 w	—	14.5	—	—	13.9 30	—	—	13.9	—	—	14.9	13.4

Table 16

Hemoglobin and Hematocrit Levels in Inositol deficient Male and Female Rats (Hb in gms./100ml. of blood (black type) relative volume of packed red cells expressed as a percent (red).

	Rat No.	Age of Rat in Weeks											
		6	8	9	12	13	14	15	17	18	19	20	23
♀	1	14.2 34	—	14.9	—	—	13.7 33	—	—	14.3 37	—	15.5	15.8
	2	—	14.9 32	—	—	14.2	—	—	13.4	—	13.3	—	12.5
	3	14.8	dead	—	—	—	—	—	—	—	—	—	—
	4	—	15.5	—	—	14.1 33	—	—	14.9 37	—	14.3	—	14.3
	5	14.1	—	13.3 36	13.4 35	—	—	14.4 41	—	13.1 38	—	14.1	13.7
♂	6	—	16.1	—	14.8 33	—	—	14.6 43	—	14.6 51	—	14.3	14.5
	7	13.0 32	—	14.8	—	—	14.3 43	—	15.3 34	—	14.8	—	14.8
	8	—	15.2 38	—	—	14.6	—	—	15.2	—	—	14.6	12.6
	9	13.6	—	14.5 37	—	—	13.4	—	—	—	13.8	—	13.4
	10	—	14.1	—	—	14.3 34	—	—	—	11.5 29	—	—	12.8

Table 17

Serum Protein Levels in B Complex Deficiencies

App. Age in Weeks	Type of Deficiency: Complete Vitamin B Complex		Thiamine		Riboflavin		Niacin		Pyrid-oxine		Calcium Panto-thenate		Choline		Inositol	
	1*	2**	1	2	1	2	1	2	1	2	1	2	1	2	1	2
4	—	—	4	5.880	—	—	—	—	—	—	—	—	—	—	—	—
5	8	6.634	—	—	4	7.416	5	6.056	5	5.744	4	6.968	—	—	—	—
6	4	6.896	4	6.652	5	6.544	—	—	—	—	—	—	5	6.652	5	6.896
7	24	6.596	2	5.808	3	6.400	4	6.056	3	6.056	—	—	—	—	—	—
8	28	5.919	7	7.380	3	7.000	—	—	—	—	3	6.336	5	7.480	5	7.720
9	—	—	5	6.160	3	6.968	4	7.212	4	6.968	—	—	5	7.528	4	7.736
10	—	—	—	—	2	7.104	2	7.528	2	7.412	4	7.036	—	—	—	—
11	—	—	—	—	2	6.512	2	7.664	4	6.896	2	7.212	—	—	—	—
12	—	—	—	—	—	—	4	7.632	4	7.732	5	6.903	2	7.384	2	7.456
13	—	—	—	—	1	7.316	2	7.416	2	7.700	2	7.560	4	7.036	4	7.288
14	—	—	—	—	1	8.560	—	—	—	—	2	8.304	4	7.732	3	7.732
15	—	—	—	—	1	8.944	4	7.800	4	7.624	2	7.280	2	7.768	2	7.664
16	—	—	—	—	1	6.584	3	7.492	4	7.360	2	6.896	—	—	—	—
17	—	—	—	—	—	—	—	—	—	—	—	—	4	8.528	4	8.528
18	—	—	—	—	—	—	—	—	—	—	—	—	4	7.764	4	7.482

1* No rats
2** Serum protein gms./100ml. sera

IV. DISCUSSION OF RESULTS

The normal results in the serum alkaline phosphatase indicate that little change occurs with development of the rat beyond three weeks. These results are in agreement with Gould (6).

The normal results for Hb and hematocrits presented here although in insufficient numbers to derive satisfactory means, are in agreement with Griffith (5) who indicates that from about 75% of the normal adult level (15.6 gms.%) the newborn rats hemoglobin slowly and irregularly increases to reach levels of 99% for the male and 103% for the female. The relative volume of packed red cells is given as 36% in the newborn rat increasing to adult levels of 49% for the male and 52% for the female. The results obtained in serum protein levels are much higher than those listed by Leathem (13) and Moore (15). These observers using Long & Evans strain rats found levels of 6 gm.%/ The difference can probably be accounted for by the two different strains of rats used.

As can be noted from the foregoing results on the effect of the deficiencies of the vitamins on the serum phosphatases, insufficient data are present to draw any satisfactory conclusions. However it might be noted that in the deficiencies of the complete vitamin B complex,

thiamine, riboflavin, niacin, pyridoxine, choline and inositol there is a lowering of the serum alkaline phosphatase toward the termination of the experiment or when the weight levels off and falls (pantothenic deficient animals remained normal). Unfortunately a weight of the food consumed was not followed and as a result one cannot correlate this fall with the intake of food but is suggestive that this is the case since the weight in every instance either levelled off or fell. Hough (11) in studying choline deficient puppies found the opposite effect to occur in the alkaline phosphatase and he recorded marked raises.

Very inconclusive results are seen with regard to the production of anemia with the deficiencies of one or all of the members of the B complex. Low results in Hb are seen in individual rats in the deficiency of the complete vitamin B complex, Ca pantothenate, pyridoxine, niacin and choline. In the last named deficiency the levels were quite low in all the animals and since the hematocrit levels too had previously been seen to be low there may be the suggestion that an anemia of a low grade was produced.

With regard to the serum protein, it appears that a lowering in the level may be indicated in thiamine and complete B deficiency.

V. SUMMARY

1. Normal development in rats has been studied in relation to the hemoglobin concentration, the hematocrit, serum protein, serum inorganic phosphatase and serum phosphatase levels and the weights.

2. The deficiencies of the complete vitamin B complex and the following members, thiamine, riboflavin, niacin, pyridoxine, choline and inositol, lower the serum alkaline phosphatase significantly. The lack of an adequate diet may account for the above fall. No alterations are seen in the serum inorganic phosphorus nor in the serum acid phosphatase.

3. The effect on the hemoglobin concentration and hematocrit levels of a deficiency of the complete vitamin B complex and the following members, thiamine, riboflavin, niacin, pyridoxine, calcium pantothenate, choline and inositol, are presented. A low grade anemia is suggested in choline deficient animals. No other significant alterations are seen.

4. Serum Protein is not altered significantly by deficiencies of the vitamin B complex produced in this experiment. However a number of instances of lowered serum protein are noted in thiamine and complete vitamin B deficient rats.

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